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TITLE: Effect of Tumor Derived TGF-β on the Efficacy of Dendritic Cell Vaccines

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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

As antigen presenting cells capable of inducing strong cytotoxic T lymphocyte (CTL) responses to specific antigens, dendritic cells (DCs) have become prime candidates for use in cancer immunotherapy. It has been shown that treatment of DCs with tumor cell supernatants results in reduced expression of MHC class II and reduced ability to induce a CTL response. These findings have led to the suggestion that tumors secrete soluble factors that inhibit the antigen presenting functions of DCs. In the clinical setting, immunization with DCs is well tolerated, but is unable to produce significant clinical responses. Taken together, these findings suggest that the tumors secrete immunosuppressive factors that interfere with the efficacy of DC immunotherapy. One such factor is transforming growth factor-ß (TGF-ß). TGF-ß is a known suppressor of T cell function and recently has been implicated in decreasing the function of antigen presenting cells. Breast cancer cells secrete TGF-ß and are less sensitive to TGF-ß mediated growth arrest. Furthermore in breast cancer patients TGF-ß immunostaining has been correlated with tumor progression. These findings suggest an important role for tumor-derived TGF-ß in the progression of mammary tumors in animals and humans. The hypothesis to be tested is that tumor-derived TGF-ß mitigates the efficacy of DC vaccines. The objective is to improve the efficacy of DC based vaccines by decreasing the suppressive effects TGF-ß has on DCs. The specific aims are to assess 1) the effect of TGF-ß on the antigen processing and presenting functions of DCs, and 2) the effect of tumor-derived TGF-ß on the efficacy of DC vaccines.

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Table of Contents

Key accomplishments

1. **Development of a method o generating functional dendritic cells**
2. **Evaluation of the effects of TGF-β on the ability of dendritic cells to process and present antigen.**
3. **Determination of the effect of TGF-β on the stimulatory function of dendritic cells**
4. **Evaluation of the effects of TGF-β neutralizing antibody on the efficacy of dendritic cell vaccines.**

Development of a method of generating functional dendritic cells

Initially, the dendritic cells (DC) used for our experimentation were generated by culturing in granulocyte macrophage colony stimulating factor (GM-CSF) alone in bacterial grade dishes. This method was selected based on the work done by Lutz et. al. (1) and the demonstration of significantly higher yields as compared to the standard method of culturing DC in GM-CSF and (interleukin-4) IL-4. Various experiments were performed to compare the functional abilities of these two cell types. Dendritic cells were evaluated for their endocytic and T cell stimulation abilities. Dendritic cells generated in the presence of GM-CSF and IL-4 were significantly more effective at endocytosing dextran particles (Table 1). Similarly dendritic cells generated in the presence of GM-CSF and IL-4 were significantly more effective at stimulating an allogeneic T cell response (Figure 1). Based on these results all subsequent experiments will be performed using dendritic cells generated in the presence of GM-CSF and IL-4.

Table 1. Endocytosis by BmDC

Culture Conditions	% Positive	Mean Flourescence Intensity
GM alone	48	146
GM +IL-4	72	756

BmDC were generated by culturing in bacterial grade petri dishes with 200 U/ml GM-CSF for 10 days (GM-alone) or by culturing in tissue culture flasks with 100 U/ml GM-CSF and 100U/ml IL-4 for 6 days (GM+IL-4) followed by the addition of 200 U/ml of TNF-α for 48 hours. Endocytosis was assessed using FITC-conjugated dextran (40,000 MW) (FITC-DX). BmDC were incubated with FITC-DX (1mg/ml) at 37°C for 30 minutes. As a negative control incubations were done at 4°C. Following incubation cells were analyzed by flow cytometry.

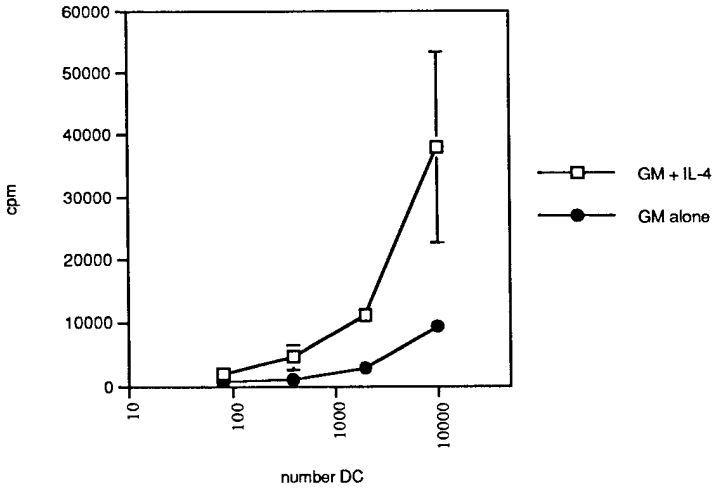

Figure 1. Stimulation of an Allogeneic Response by BmDC. BmDC were generated as indicated above from Balb/cJ mice. BmDC were incubated for 5 days with $2x10^5$ allogeneic splenic T cells isolated from C57BL/6 mice. Cells were pulsed with [^{3}H] thymidine for the last 18 hours of culture. Numbers are mean ± SEM of triplicate samples

Evaluation of the effects of TGF-β on the ability of dendritic cells to process and present antigen.

To evaluate the effect of TGF-β on DC antigen processing and presentation, DC were generated and cultured in TGF-β for 48 hours and then analyzed for their ability to stimulate the proliferation of allogeneic cells in a mixed lymphocyte reaction (MLR) (Figure 2). Pretreatment of DC with TGF-β did not significantly affect the ability to induce the proliferation of allogeneic cells. To test the effects of TGF-β on antigen processing and presentation, DC were pretreated with TGF-β and pulsed with ovalbumin (OVA) protein and evaluated for their ability to induce to interleukin-2 (IL-2) production by the OVA specific T cell hybridoma (DO11.10). DO11.10 cells recognize OVA peptide and respond by secreting IL-2 (Figure 3). Pretreatment of DC with TGF-β did not significantly affect the ability to process and present OVA protein to DO11.10 hybridoma cells.

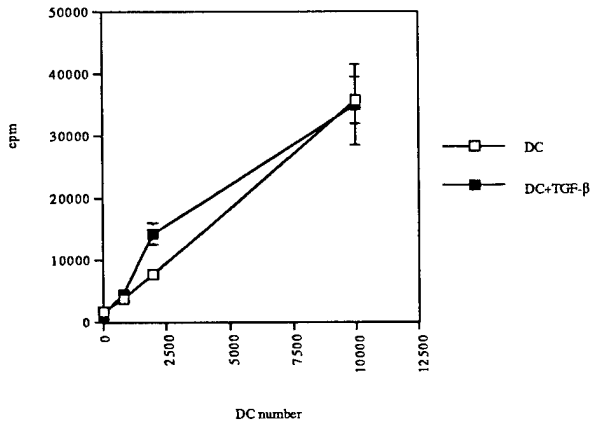

Figure 2. TGF-β does not inhibit the ability of DC to stimulate the proliferation of allogeneic cells. BmDC were generated from Balb/cJ mice by culturing in tissue culture flasks containing 100 U/ml of GM-CSF and IL-4. Following 6 days of culture 2 ng/ml of TGF-β was added for 48 hours. BmDC were collected, washed and an allogeneic MLR was performed as described above. Numbers are mean ± SEM of triplicate samples. Data is representative of three independent experiments.

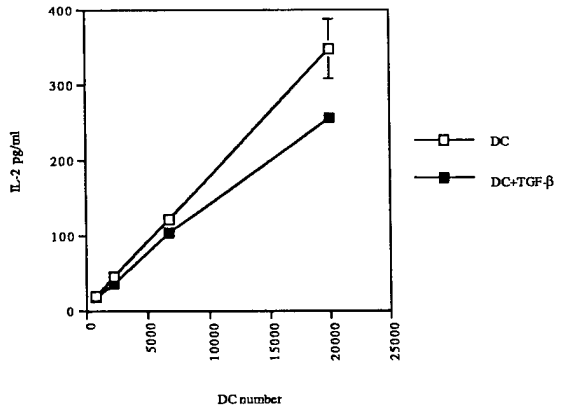

Figure 3. TGF-β does not inhibit the ability of DC to process and present OVA to DO11.10 hybridoma cells. BmDC were generated and treated with TGF-β as indicated above. BmDC were collected, washed and incubated with 1 mg/ml ovalbumin protein and 105 DO11.10 cells for 48 hours. Supernatant was analyzed by ELISA for IL-2 production. Numbers are mean ± SEM of triplicate samples. Data is representative of three independent experiments.

Determination of the effect of TGF-β on the stimulatory function of dendritic cells

We have also evaluated the effect of TGF-β on the ability of DC to stimulate T cell responses. This was achieved using a proliferation assay in which tumor lysate-pulsed DC were co-incubated with syngeneic purified splenic T lymphocytes isolated from naive mice (Figure 4A) or mice bearing 4T1 tumors (Figure 4B). The ability of DC to stimulate the proliferation of T cells from naïve mice was significantly inhibited ($p=0.0147$) when DC were treated with TGF-β. Maturation of DC, by tumor necrosis factor -α (TNF-α) abrogated the suppressive effect of TGF-ß. However, mature DC were most effective at stimulating in vivo tumor-sensitized T lymphocytes from tumor-bearing mice (Figure 2B). Interestingly, unlike the case with naïve T cells, TGF-ß treatment significantly suppressed the ability of mature DC to present antigen to tumor-sensitized T cells.

Antigen (tumor lysate)-pulsed DC were also evaluated for the ability to induce the production of a Th1 cytokine, interferon-γ (IFN-γ) by T cells isolated from lymph nodes draining 4T1 tumors. Treatment with TGF-β significantly inhibited the ability of both immature and mature DC to stimulate IFN-γ secretion by T lymphocytes (Figure 5). Taken together, these data demonstrate that TGF-ß can inhibit the ability of DC to stimulate tumor-sensitized T cells and result in diminished production of Th1 cytokines such as IFN-γ involved in cell-mediated tumor immunity.

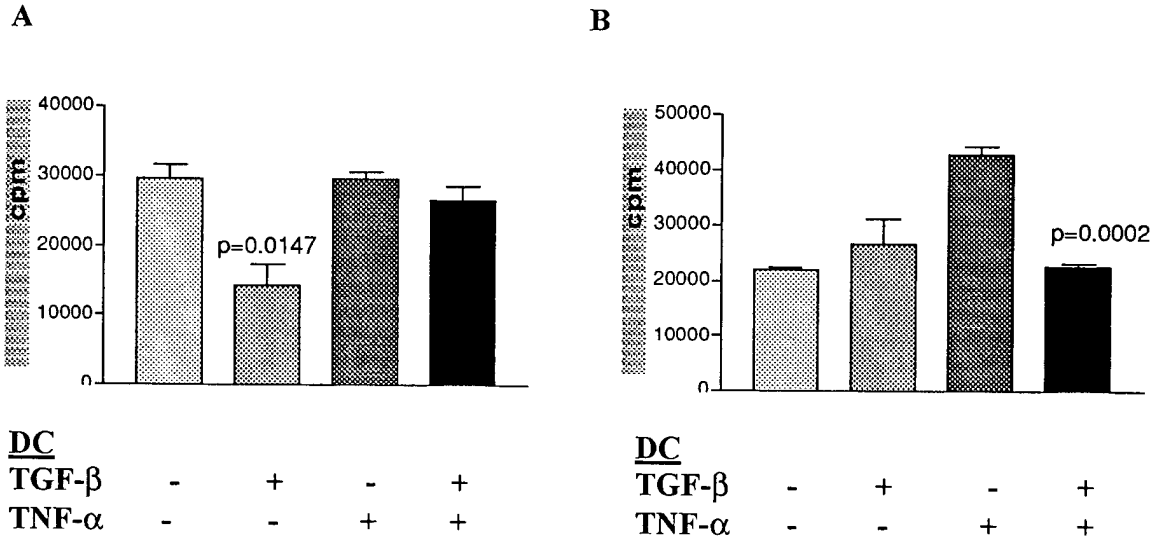

Figure 4. Effect of TGF-β on BmDC ability to stimulate T cell proliferation. BmDC were generated as described above. Following 6 days of culture in GM-CSF and IL-4, BmDC were pulsed with 4T1 cell lysate for 48 hours in the presence or absence of 10ng/ml of TGF-β, then cultured with TNF-α in the presence or absence of TGF-β for 48 hours. Ten thousand DC were then incubated with $2x10^5$ splenic T cells isolated from naive mice (**A**) or mice bearing 14 day 4T1 tumors (**B**) for 4 days and pulsed with [^{3}H] thymidine for the last 18 hours of culture. Numbers are mean ± SEM of triplicate samples.

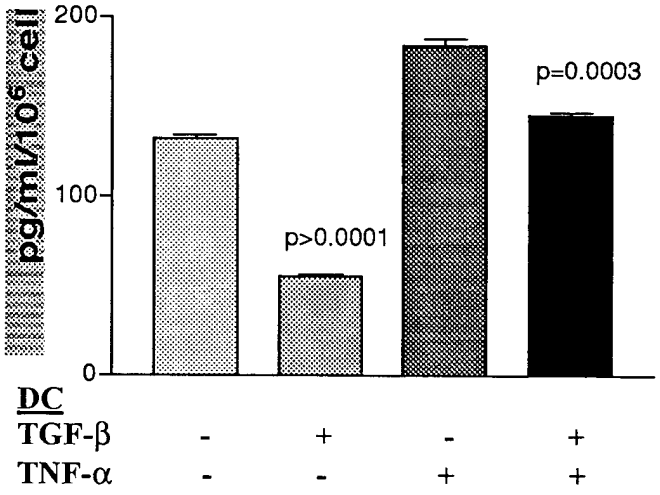

Figure 5. Effect of TGF-β on BmDC-induced IFN-γ production by T cells. BmDC were generated and treated with TGF-β and TNF-α as described above. One million T cells isolated from lymph nodes draining 14 day 4T1 tumors were incubated with $2.5x10^5$ BmDC for 48 hours. Following incubation supernatant was analyzed for IFN-γ production by ELISA. Numbers are mean ± SEM of triplicate samples.

Evaluation of the effects of TGF-β neutralizing antibody on the efficacy of dendritic cell vaccines.

A preliminary experiment was performed to determine if neutralizing intratumoral and systemic TGF-ß in tumor-bearing animals would have an impact on DC vaccination. To address this issue tumors were injected 2 times with tumor-lysate pulsed DC plus i.t. and i.p. TGF-ß-neutralizing antibody (2G7). The data shown below demonstrate that tumor growth continued in all 3 mice treated with DC alone, or with DC plus matched isotype control antibody. In contrast, tumor inhibition was observed in 2 of 3 tumors in animals vaccinated with DC plus 2G7. In order to substantiate these observations the experiment will be repeated with the addition of mice treated with 2G7 alone and mice treated with isotype antibody alone.

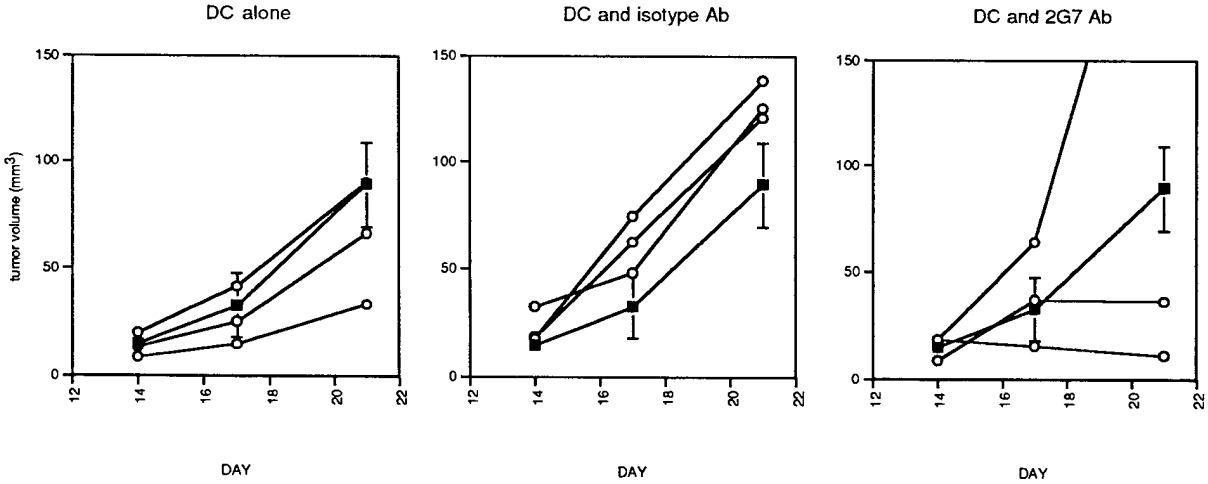

Figure 6. Effect of TGF-β neutralizing antibody on DC vaccination. Mice with established 4T1 tumors were vaccinated on day 14 and day 18 with 10^6 tumor lysate-pulsed DCs alone or in combination with 200μg i.t. and 200μg i.p 2G7 or isotype control antibody and monitored for tumor growth. Solid squares represent average tumor volume of untreated mice. Open circles represent tumor volume of individual mice.

Reportable Outcomes

Funding applied for and received based on research supported by this grant
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"Tumor-Mediated Suppression of Dendritic Cell Vaccines"

Presentations
Research supported by this grant will be included in a poster to be presented at the 16th annual Society for Biological Therapy Meeting in Washington, DC on November 16th-19th 2001

References

1. Lutz, M.B., N. Kukutsch, A. L. J. Ogilvie, S. Roβner, F. Koch, N. Romani and G. Schuler. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Meth. 223:77-92, 1999.